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Effect of Temperature of Incubation on Activation of Brain-Prothoracic Gland System of Diapausing Pupae in Samia cynthia pryeri

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Abstract

Termination of diapause occurs with activation of brain-prothoracic gland system in diapausing pupae of *Samia cynthia pryeri*. It does not occur with 20°C incubation, but it occurs with 26°C incubation. To investigate the cause of this fact histological change of neurosecretory cells of pupal brain during incubation and effect of β-ecdysone injection were observed. In histological observation, phloxinophilic granules of "lateral B type cells" in pars intercerebralis decreased on and after the 5-th day of 20°C incubation. Diapausing pupae developed into imagines with β-ecdysone injection during 20°C incubation. From these results, it is concluded that fail of termination of diapause with 20°C incubation is not resulted from the inactive state of neurosecretory cells in brain but it is resulted from the inactive state of ecdysone secretory system in this temperature.

Introduction

The mechanism of pupal diapause was studied by WILLIAMS, C. M. on the diapause of cecropia silkworm, *Hyalophora cecropia* ('46, '47, '52). He demonstrated the following fact that onset and termination of the pupal diapause of cecropia silkworm were resulted from the decrease and increase of the hormonal activity of "brain-prothoracic glands system". It was also reported that pupal diapause of another wild silkworm, *Samia cynthia pryeri*, was controlled by the similar mechanism with that of cecropia silkworm (KOENUMA, '85).

It is possible to induce termination of pupal diapause in laboratories when diapausing pupae of *Samia cynthia pryeri* were incubated in 26°C after long period of storage with 5°C. However, rate of imagination was always higher

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in the pupae which were reared in outdoor environment than the pupae reared in laboratories. In connection of these facts, it was reported a remarkable increase of rate of imagination took place in the case in which chilled pupae were incubated with 26°C after over 15 days of 20°C preincubation (KOENUMA '85). However, when the chilled pupae were incubated with 20°C for 45 days, the pupae did not grow into imagines. With the cause of this fact, following possibilities were considered: (1) Prothoracicotropic hormone would not be secreted from neurosecretory cells with 20°C incubation, (2) Prothoracic gland would not secret the hormone with this temperature, (3) Both brain and prothoracic gland would be activated with this temperature but target tissue of the prothoracic gland hormone would not react with the hormone. This study was carried out to attempt to investigate each of these possibilities.

Material and methods

Pupae used in this study were pupae of *Samia cynthia pryeri*. Before incubation they were kept 20°C for 4 weeks from their pupation and then they were kept in a refrigerator with 5°C for over 60 days.

Following experiments were performed.

(1) Observation of change of lateral B type neurosecretory cells in pars intercerebralis of pupal brain during 20°C incubation. The lateral B type neurosecretory cells in pars intercerebralis were observed with GOMORI's chrome-haematoxilin phloxin staining method in $10^{\mu m}$ thick paraffin sectioning samples.

(2) Injection of β-ecdysone to chilled pupae during incubation. In the one series of experiments, various doses of β-ecdysone were injected to the chilled diapausing pupae just before start of incubation. In these experiments, both 20°C incubation and 26°C incubation were employed respectively.

In the other series of experiments, each $8^{\mu g}$ β-ecdysone per gram weight of pupa determined from the proceeding experiments was injected during various periods of incubation to the two groups of pupae. In these experiments, each group consisted of intact pupae and decerecrated pupae respectively. The time of injection was just before start of incubation, after 5 days of incubation, after 10 days of incubation, after 15 days of incubation and after 20 days of incubation. The temperature of incubation was 20°C and 26°C respectively. The extirpation of brain was carried out at the same time with injection. In the case in which 26°C incubation was employed, both injection experiments of the decerebrated pupae and injection at the 21-st day of incubation were omitted.

β-ecdysone used was made in Rohoto Pharmacial Co. Ltd.. It was prepared for injection medium to dissolve in EPHRUSSI BEADLE's salt solution which

contain 4 to $12^{\mu g}$ per gram pupal weight of β-ecdysone, then $10^{\mu l}$ of it was injected into the dorsal side of the 2-nd or 3-rd abdominal segment of each pupa. In control experiment, $10^{\mu l}$ of EPHRUSSI BEADLE's salt solution without β-ecdysone was injected.

The pupae injected were observed continuously for 45 days. Development was evaluated with following criteria:

(1) pigmentation of pupal cuticle
(2) formation of adult legs
(3) formation of wing tissue
(4) formation of compound eyes
(5) formation of tentacles
(6) pigmentation of wing
(7) pigmentation of abdomen
(8) filling of ecdysial sap
(9) completion of pharete moth

Of these nine stages, each stage was evaluated with one point as complete appearance, half point as incomplete appearance and zero as without appearance respectively. If the sum of them was 9 points, then it was regarded as normal development. If the sum of them was between 0.5 point and 8.5 points, it was regarded as abnormal development. And if the sum of them was zero, it was regarded as no-deveropment.

Results

(1) *Observation of change of the lateral B type neurosecretory cells in pars intercerebralis of pupal brain during 20°C incubation*

Fig. 1 shows the lateral B type neurosecretory cells in pars intercerebralis

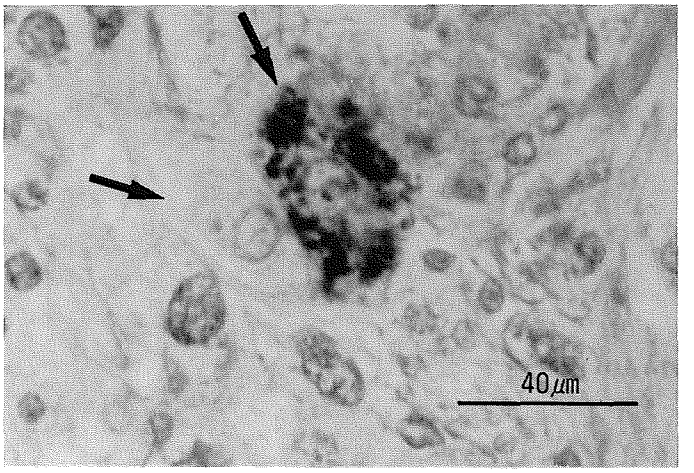

Fig. 1 lateral B type neurosecretory cells in pars intercerebralis of brain of the pupa kept for 60 days in 5°C

of brain of the pupa chilling for long perieod in 5°C. In this figure, the lateral B type cells are indicated by arrows. They are filled by large quantity of phloxinophilic granules.

These granules are derceasing in the third day of 20°C-incubation shown in Fig. 2.

Fig. 3 shows the lateral B type neurosecretory cells in pars intercerebralis of brain of the pupa incubated for 5 days with 20°C incubation. The lateral B type cells are indicated by arrows in this figure. It is shown in this figure the phloxinophilic granules in lateral B type cells almost disappear.

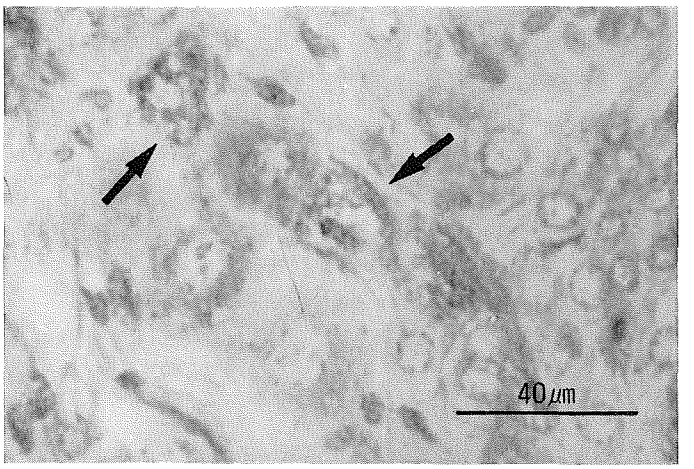

Fig. 2 lateral B type neurosecretory cells in pars intercerebralis of brain of the chilled pupa on the second day of the 20°C

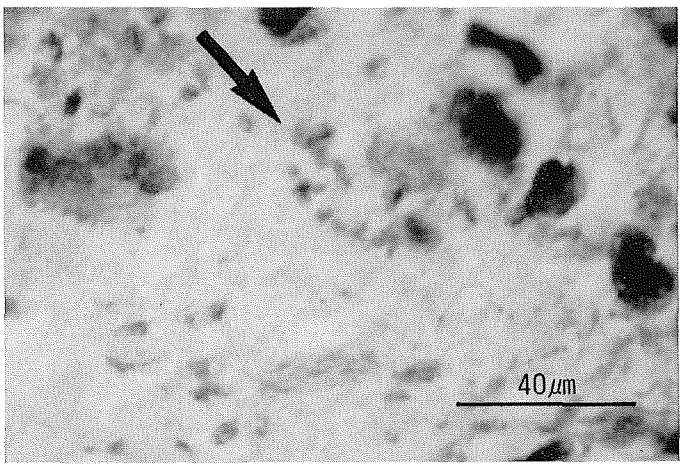

Fig. 3 lateral B type neurosecretory cells in pars intercerebralis of brain of the chilled pupa 5 days after beginning of 20°C incubation

Lateral B type neurosecretory cells in pars intercerebralis of the pupae incubated for 10 days and 15 days with 20°C incubation are shown in Fig. 4 and Fig. 5 respectively. In these figures the lateral B type cells are indicated by arrows. They are all empty with phloxinophilic granules.

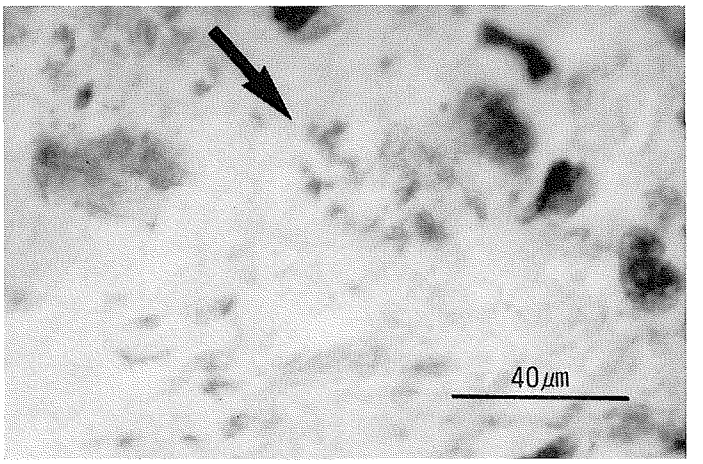

Fig. 4 lateral B type neurosecretory cells in pars intercerebralis of the brain of the pupa on the 10-th day of 20°C incubation

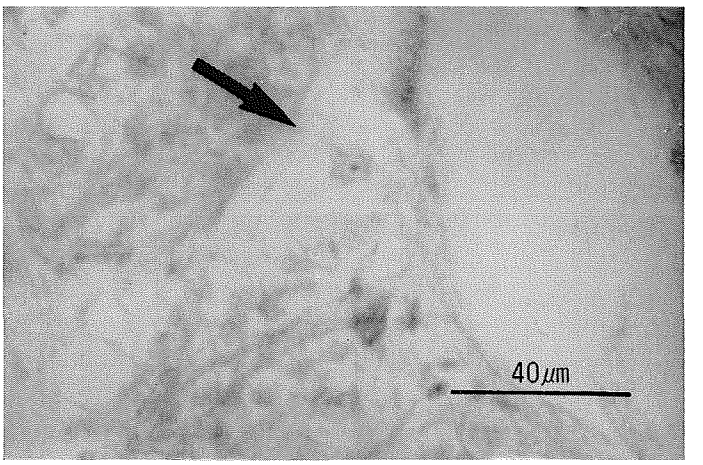

Fig. 5 lateral B type neurosecretory cells in pars intercerebralis of the chilled pupa on the 15-th day of 20°C incubation

(2) *Results of β-ecdysone injection to the chilled pupae during incubation*

Results of β-ecdysone injection with various doses to the chilled pupae at the time of transference into 20°C incubation or 26°C incubation are present in Fig. 6 and Table 1. As being shown with a broken line, the pupae reared in 26°C after the injection are activated with the doses higher than $4^{\mu g}$/g. But

Table 1 Effect of β-ecdysone on diapausing pupae of *S. cynthia pryeri*

temp. incubation	dose μg/g	No. of pupae	development		
			normal	abnormal	fail
26°C	0	8	0	0	8
26°C	4	6	4	1	1
26°C	6	16	6	5	5
26°C	8	18	5	10	3
26°C	10	16	3	13	0
26°C	12	12	0	11	1
20°C	0	8	0	0	8
20°C	4	10	1	2	7
20°C	6	12	6	4	2
20°C	8	15	10	3	2
20°C	10	18	7	9	2
20°C	12	12	6	6	0

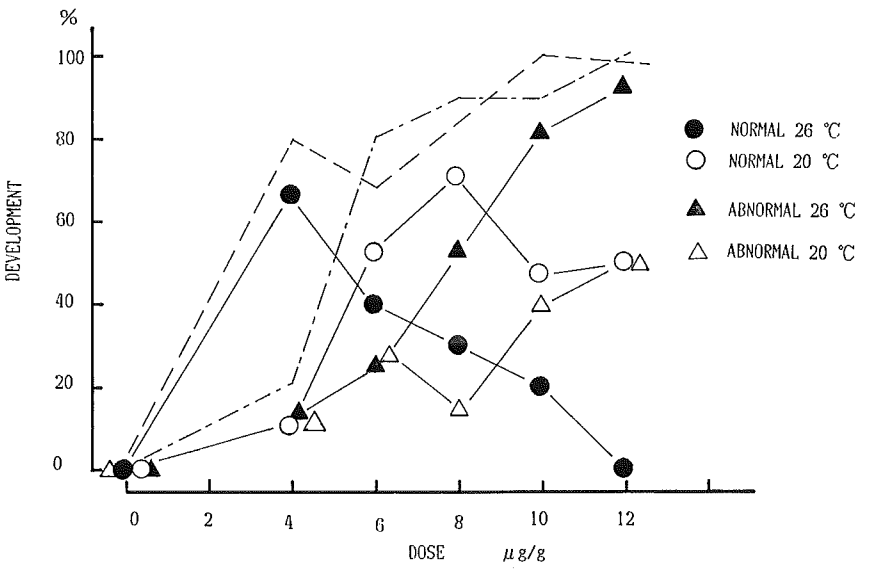

Fig. 6 development of pupae which are injected various dose of β-ecdysone

the rate of normal development of them which is represented by solid circle is maximal with the dose of 4μg/g, and the rate of normal development becomes lower and lower as the dose becomes higher and higher. In this temperature, the rate of abnormal development which is represented by solid triangle increases as the dose of β-ecdysone increases. In 20°C incubation, the rate of

normal development wich is represented by hollow circle decreases with the dose of β-ecdysone higher than 8$^{\mu g}$ per gram weight of the pupa, and the rate of abnormal development increases gradually with the dose of β-ecdysone higher than that.

Results of β-ecdysone injection experiments to the pupae at various periods during incubation are present in Fig. 7 and Table 2. In these experiments,

Table 2 Effect of β-ecdysone on chilled pupae of *S. cynthia pryeri*

operation	incubation temperature	duration of 20°C	dose	No. of pupae	development		
			μg/g		normal	abnormal	fail
		days					
intact	26°C	0	8	15	8	7	0
intact	26°C	5	8	7	2	5	0
intact	26°C	10	8	7	0	7	0
intact	26°C	15	8	7	7	0	0
intact	20°C	0	8	7	0	4	3
intact	20°C	5	8	7	5	2	0
intact	20°C	10	8	7	2	5	0
intact	20°C	15	8	7	7	0	0
intact	20°C	20	8	6	6	0	0
intact	20°C	0	0	8	0	0	8
−Br	20°C	0	0	8	0	0	8
−Br	20°C	0	8	7	7	0	0
−Br	20°C	5	8	7	7	0	0
−Br	20°C	10	8	7	7	0	0
−Br	20°C	15	8	7	7	0	0

injection is carried out in the following three cases: that is, the first case in which chilled pupae are incubated with 20°C then they are reared in 20°C after the injection at various periods of incubation, the second case in which the pupae are reared in 26°C after the injection, and the third case in which the pupae are reared in 20°C after both injection and the extirpation of brain at the same periods of incubation. The dose of β-ecdysone is 8$^{\mu g}$ per gram weight of each pupa. In Fig. 7 each symbol represents as following: hollow circle as the rate of normal development with 20°C incubation, solid circle as the rate of normal development with 26°C incubation, hollow square as the rate of normal development of the decerebrated pupa with 20°C incubation, hollow triangle as the rate of abnormal development with 20°C incubation, solid triangle as the rate of abnormal development with 26°C incubation and cross as the rate of

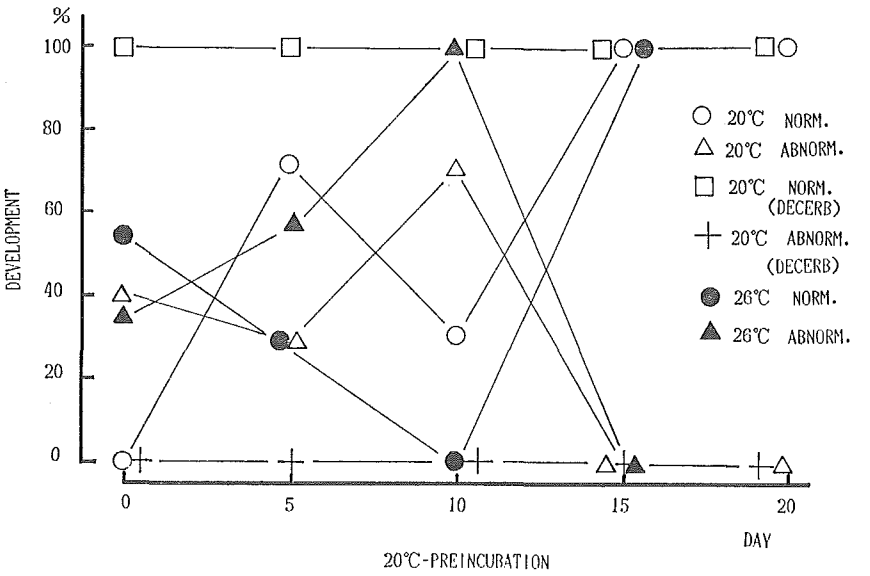

Fig. 7 rate of imagination obtained from the β-ecdysone injection experiments

abnormal development of the decerebrated pupa with 20°C incubation. In the first case, the rate of normal development reveals in about 70% when the injection is carried out at the 5-th day of incubation, and it becomes in 100% when the injection is carried out at the 15-th day of incubation and after. But when the injection is carried out at the 10-th day of incubation, the rate of normal development decreases. In this case, the rate of abnormal development has a tendency which decreases from about 40% in the first day injection to zero in the 15-th day injection, but in the 10-th day injection it increases to about 70 %. In the second case, the rate of normal development decreases from about 55% in the first day injection to zero in the 10-th day injection, then it increases to 100% in the 15-th day injection. In this case, the rate of abnormal development increases from about 35% in the first day injection to 100% in the 10-th day injection, then it decreases to zero in the 15-th day injection. In the last case, the rate of normal development is maintained in 100% regardless of the period of injection, and the rate of abnormal development is also maintained in zero regardless of the period of injection.

Discussion

Histological changes corresponding to termination of diapause of the lateral B type neurosecretory cells of pars intercerebralis of brain of pupa of *Samia cynthia pryeri* have been reported by KOENUMA ('85). He concluded that the

lateral B type cells of pars intercerebralis were the source of the prothoracicotropic hormone of brain, and their histological features with deficient in phloxinophilic granules were the characteristic of the cells secreting hormone. In this experiment in which chilled pupae were incubated with 20°C, phloxinophilic granules of lateral B type cells of pars intercerebralis decreased in the third day of incubation, and the feature with deficient in phloxinophilic granules continued throughout 20°C incubation. This fact seems to suggest that prothoracicotropic hormone is released with 20°C incubation of chilled pupae as well as with 26°C incubation.

It has been reported that the pupae which were chilled for long period failed to develop to imagines when they were incubated with 20°C. It was considered as the one of possible causes of this failure of development that no release of prothoracicotropic hormone of brain occurred with 20°C incubation. However, the result of this histological observation of lateral B type cells of pars intercerebralis with 20°C incubation is not coincident with this possibility and the brain seems to activate to release hormone with 20°C incubation. Therefore, the cause of failure of development with 20°C incubation does not seem the failure of release of hormone from brain.

It has been reported the second possible cause of the failure of development with 20°C incubation of chilled pupae that no hormone was released from the prothoracic glands in spite of release of prothoracicotropic hormone from brain in this temperature (KOENUMA, '85). It has also been reported the third possibility of the failure of development that prothoracic gland hormone is released during 20°C incubation, but the target of the hormone has no reactivity to the hormone (KOENUMA, *l. c.*). In these experiments, termination of diapause and imagination of pupae were resulted from the injection of β-ecdysone in both 20°C incubation and 26°C incubation, and imagination of pupae was also resulted from the injection of β-ecdysone to the decerebrated pupae in 20°C incubation. This latter result is contradictory with the third possibility of the cause of failure of development of pupa. Therefore, failure of development of pupa during 20°C incubation is attributable to the possibility that no β-ecdysone act to the targets, for prothoracic glands remain inactive in 20°C although activation of brain occurs in this temperature. However, it cannot immediately conclude from only these results of the injection of β-ecdysone that release of the hormone from prothoracic glands is not induced by the action of prothoracicotropic hormone of brain during 20°C incubation. There is a report that the hormone released from the prothoracic glands which are activated by prothoracicotropic hormone of brain is not β-ecdysone but α-ecdysone (CHINO et al. '74). A possibility that the failure of development of pupa in 20°C incubation

would be derived from the failure of modification of α-ecdysone to β-ecdysone still remains from only the results of the injection of β-ecdysone. Therefore, it may be required the experiments of injection of α-ecdysone.

MEOLA, R.W. and ADKISSON, P.L. ('77) reported that termination of diapause of pupae of *Heliothis zea* did not occur in 21°C though prothoracicotropic hormone was released within 24 hours after the larval-pupal ecdysis, it occurred in either the case where the pupae were transferred into a high temperature (27°C) or injected α-ecdysone in 21°C. Here is a similar fact with the fact of this case in the pupae of *S. cynthia pryeri.*

A tendency in the increase of abnormal development was shown in the experiments of injection of β-ecdysone to the chilled pupae between the beginning day and the 10-th day of 20°C incubation, but this tendency was not shown in the decerebrated pupae. This fact suggests that there is another function that induces the abnormal development of pupa in early period of 20°C incubation. This function seems to exist in the pupal brain, and it seeme to increase gradually till the 10-th day of incubation and decrease rapidly after that period.

Acknowledgiment

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